# THE OTHER FLUORESCENCE(S)

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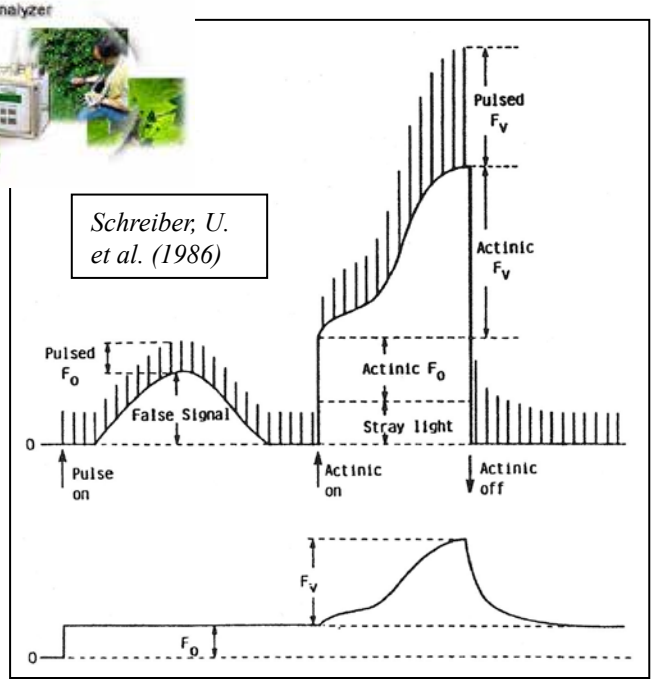
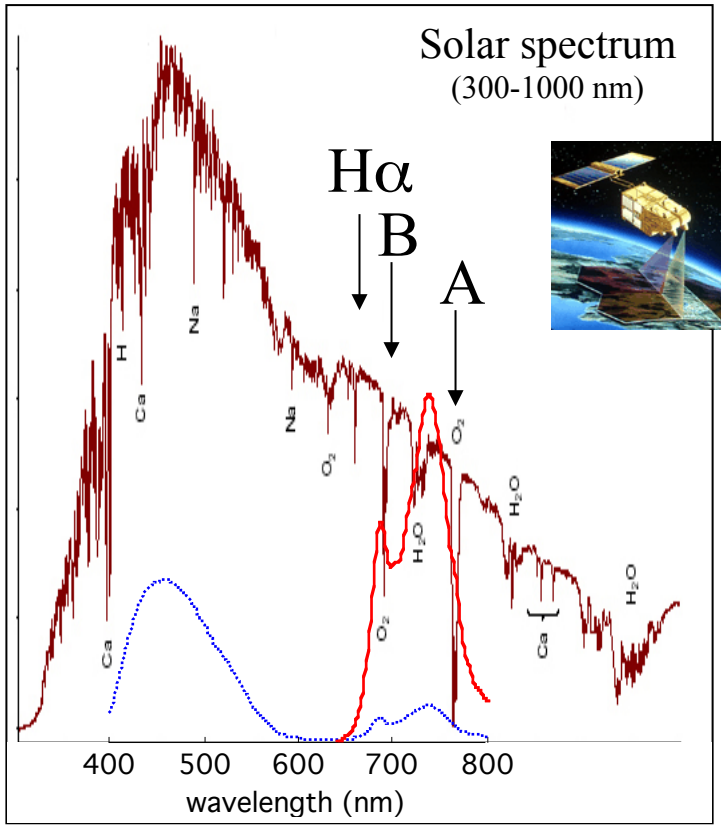
# The **variable** chlorophyll fluorescence **"Dynamic fluorescence"**

**Active fluorescence**
linked to photosynthesis
and stress
Kautsky kinetics
Quenching analysis
*Ulrich Schreiber* (PAM)


**Passive fluorescence**
linked to gross primary production
**Sun-induced fluorescence** (SIF)



# Avoiding the variable chlorophyll fluorescence **"Static fluorescence"**

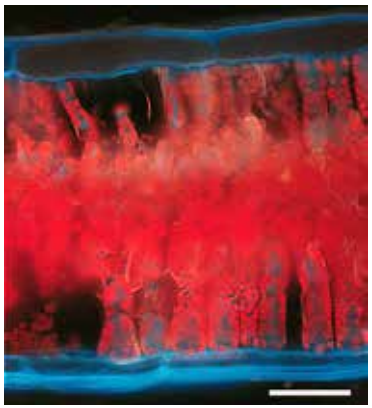
**Fluorescence linked to plant constituents**

*Fluorescence spectral ratios:*

Fluorescence excitation ratio (FER),
Simple fluorescence emission ratio (SFR),
Nitrogen balance index (NBI).

**UV-induced blue-green fluorescence (autofluorescence)**

Blue-to-red emission ratio (BRR)
UV-induced violet-blue fluorescence (VBF)



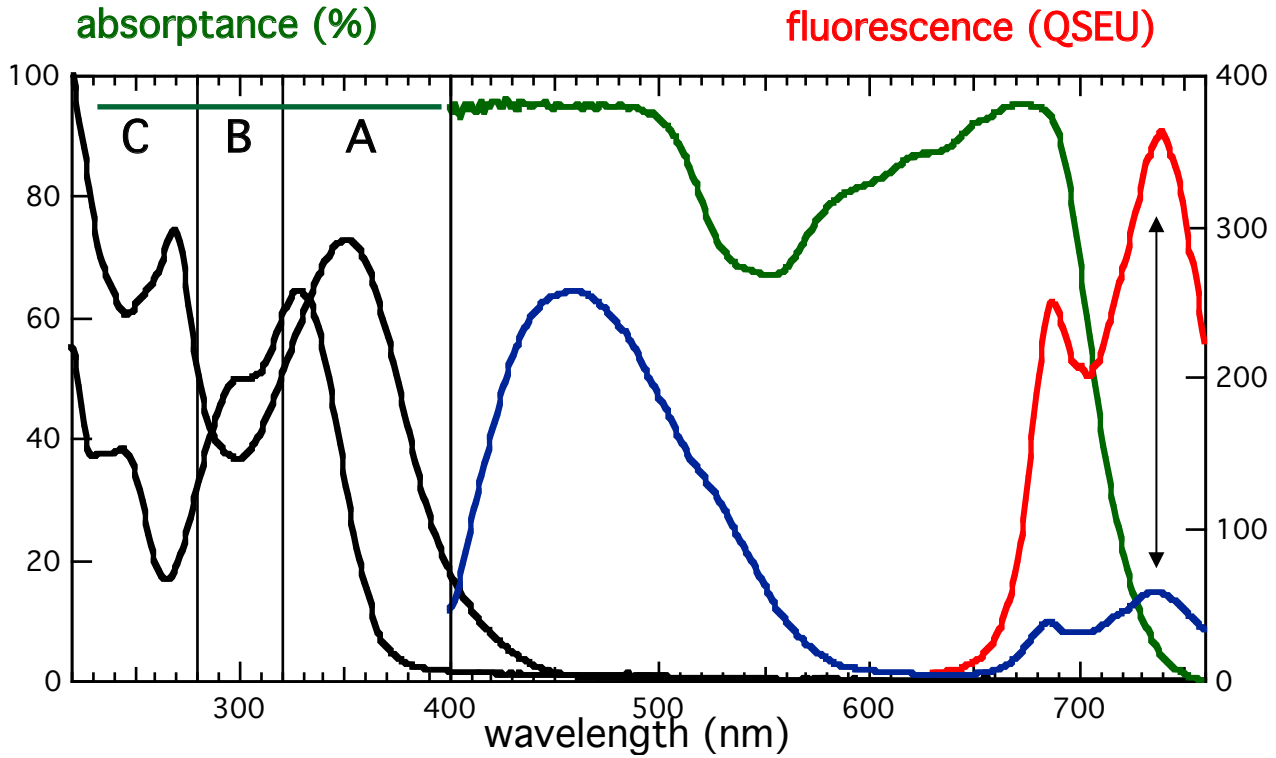
# Leaf optical properties in the UV-VIS

In the range 220 - 360 nm
Transmittance = 0.24 - 0.32 %
Reflectance = 4.2 - 5.8 %

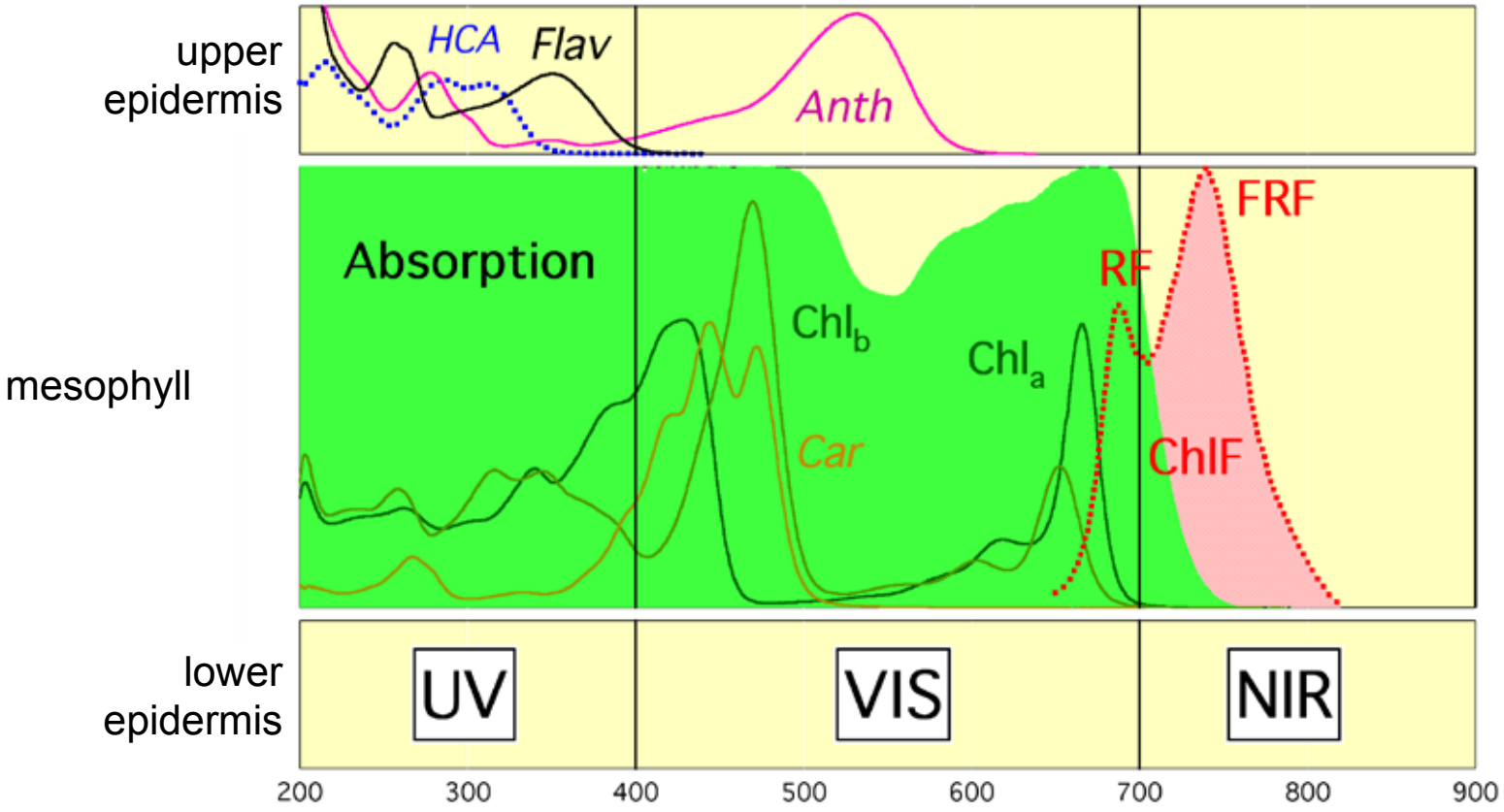
For 10 crops

*Gausman et al. (1975)*
*Rodriguez & Gausman (1977)*
*Grant et al. (2003)*

UV-B
T = 0 %
R = 5 %



# Chlorophyll fluorescence screening & reabsorption

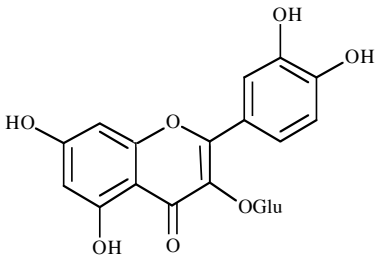



*Cerovic et al. (1999) Agronomie,* ***19:*** *543*

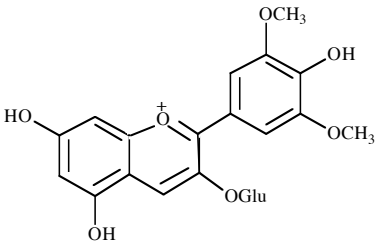
# The chlorophyll fluorescence excitation ratio (FER)

**The use of chlorophyll fluorescence to estimate the content of flavonoids in leaf epidermis**

A method based on excitation screening by **flavonols** or **anthocyanins**

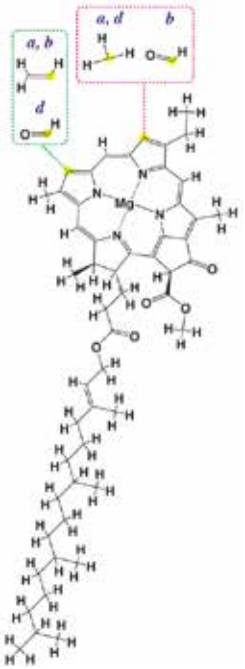



*Quercitrin*




*Oenin*

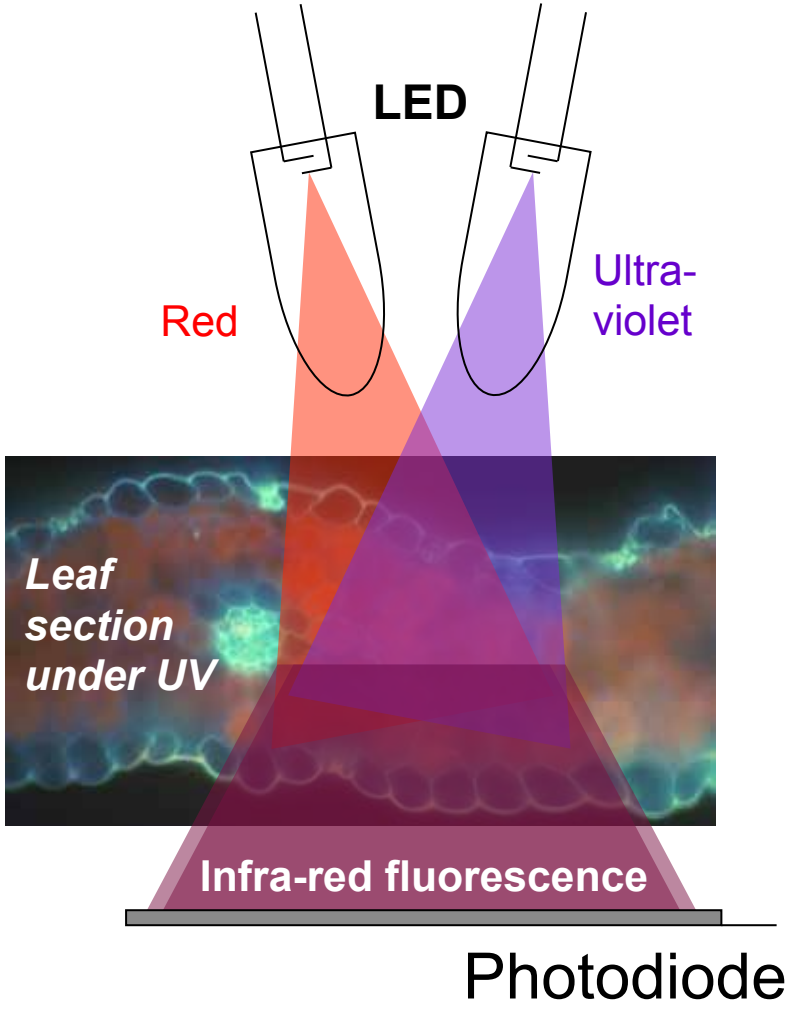
*Chlorophyll a & b*



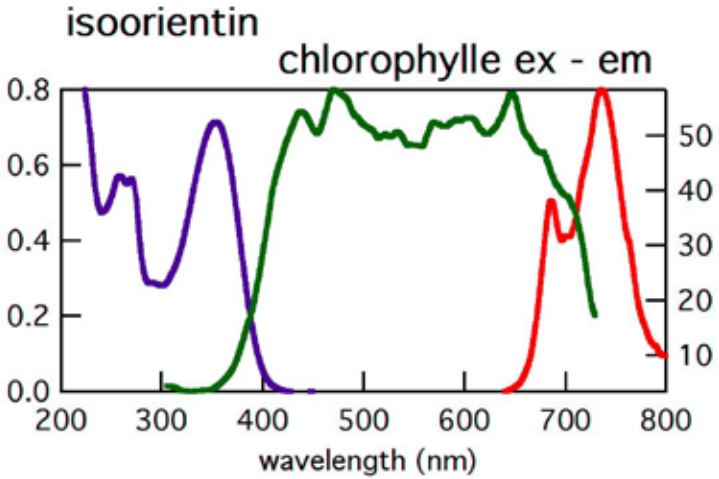

Two indices: FLAV and ANTH

Log of the chlorophyll fluorescence excitation ratio

# Dualex FLAV based on ChlF screening method




Goulas et al. (2004) Applied Optics **43**, 4488

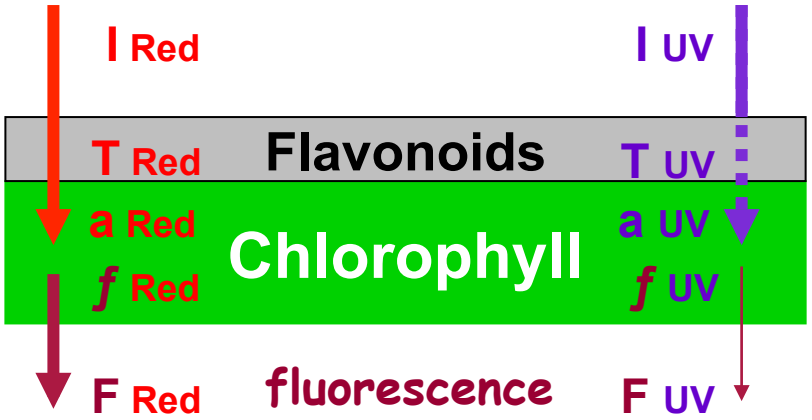



**Flavonoids are present in the epidermis. They absorb UV radiation and screen the mesophyll.**

**Chlorophyll from the mesophyll emits near-IR fluorescence measurable on both sides of the leaf.**

# The Dualex measuring principle

*Goulas et al., Applied Optics **43**, 4488*




**I Red = RED LIGHT EXCITATION**
**I UV = UV RADIATION EXCITATION**

**I = IRRADIANCE**
**T = EPIDERMAL TRANSMITTANCE**
**a = MESOPHYLL ABSORPTANCE**
***f*** **= FLUORESCENCE YIELD**
**F = CHLOROPHYLL FLUORESCENCE**

$$\frac{F_{Red} = I_{Red} * T_{Red} * a_{Red} * f_{Red}}{F_{UV} = I_{UV} * T_{UV} * a_{UV} * f_{UV}}$$

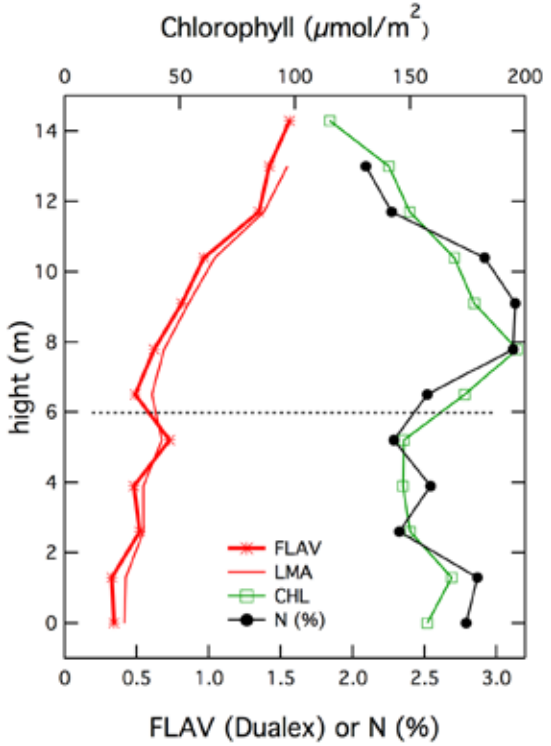
for $T_{Red} = 1$

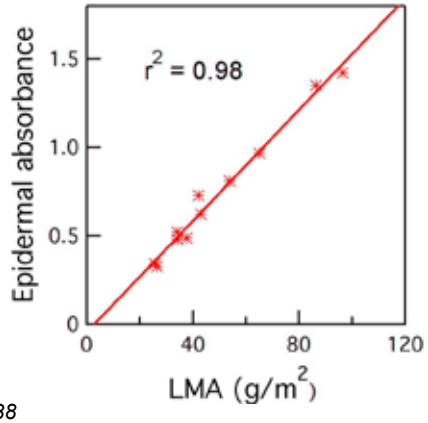
and $\frac{I_{Red} * a_{Red} * f_{Red}}{I_{UV} * a_{UV} * f_{UV}}$ = constant

$$\log \frac{F_{Red}}{F_{UV}} = \log \frac{1}{T_{UV}} + c = A_{Flav} + c$$

# FLAV index: a integrated irradiance meter

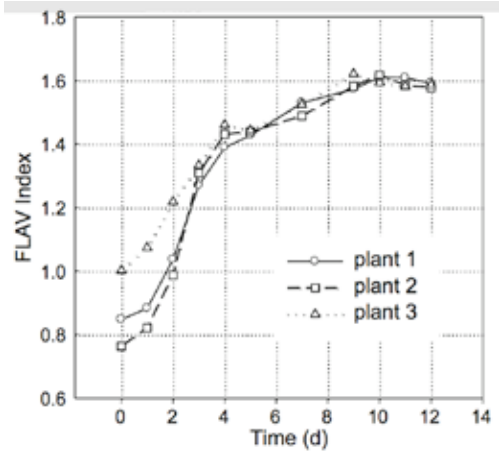
## Hesse forest

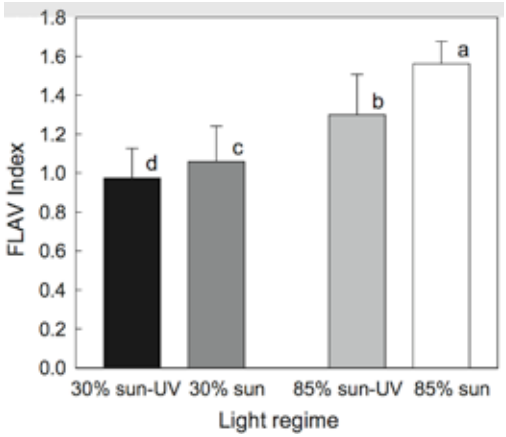



*Meyer et al.(2006) Plant Cell Environ.* ***29****: 1338*

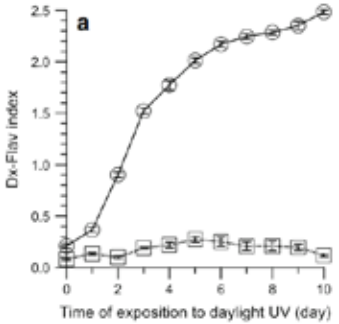
*Agati et al. (2011) Environ. Exp. Bot.* ***73****: 3*

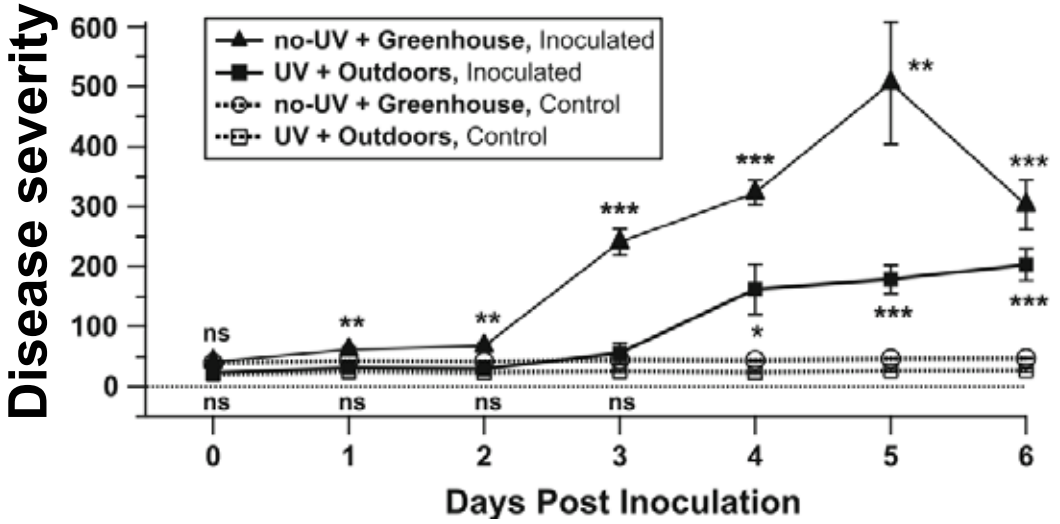


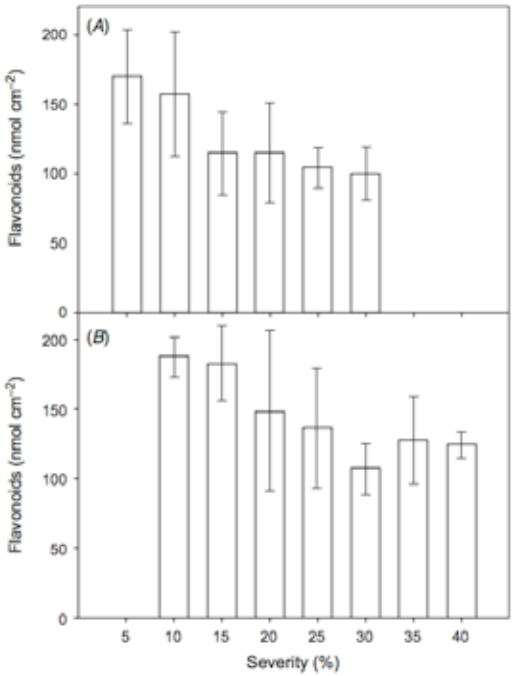



# FLAV index: an indicator of susceptibility to diseases

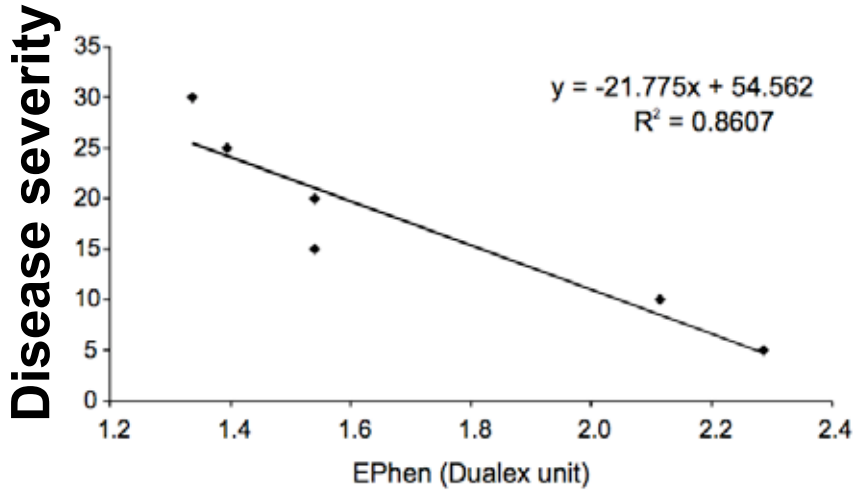
*Latouche et al.(2013) Planta **237**: 351*



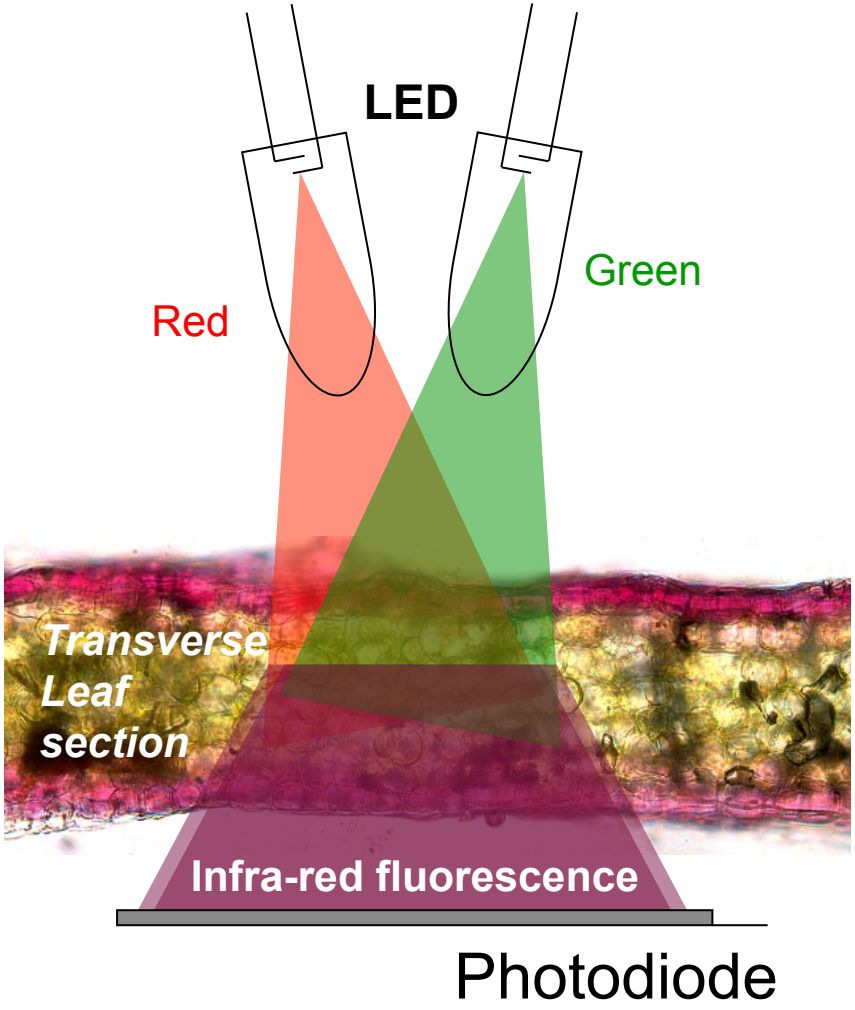




*Agati et al. (2008) Funct. Plant Biol. **35**: 77*

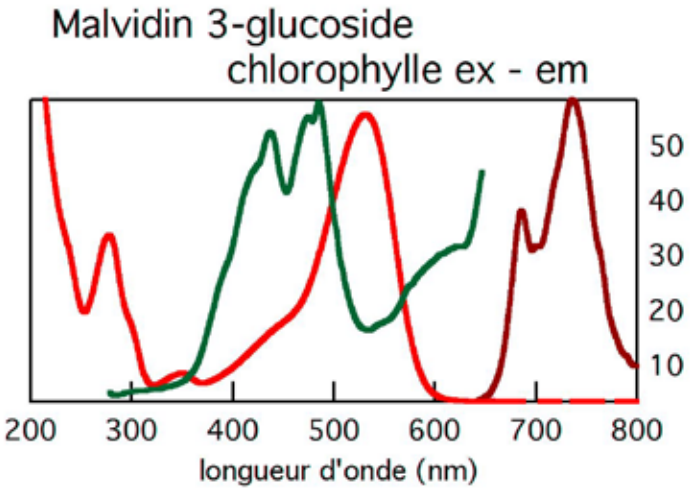



*Dalla Marta et al. (2008) Sci. Agric. (Piracicaba, Braz.) **65**: 65*

# The Dualex ANTH index




*acc. Goulas et al. (2003) Patent WO03029791*




**When anthocyanins are present in the epidermis they absorb green light and screen the mesophyll.**

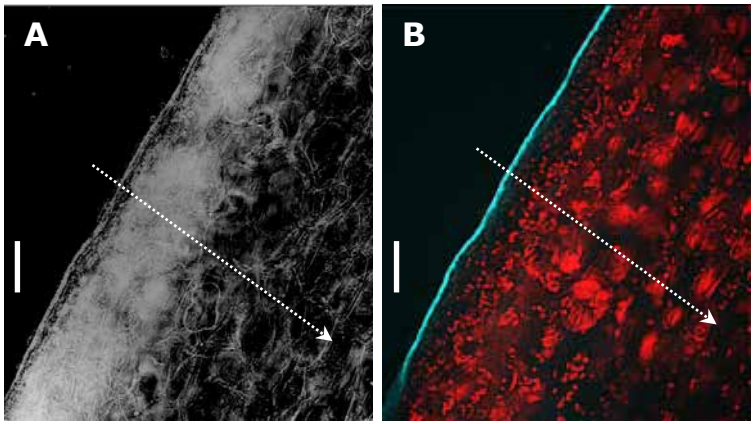
**Chlorophyll from the mesophyll emits near-IR fluorescence measurable on both sides of the leaf.**

# Chlorophyll fluorescence excitation screening in berries

*Agati et al. (2007) JAFC, **55**, 1053*

Transmission (546 nm)

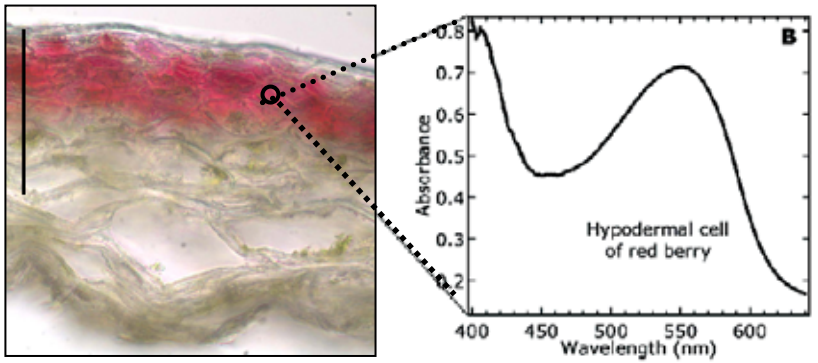
UV-excited
Fluorescence (680 nm)

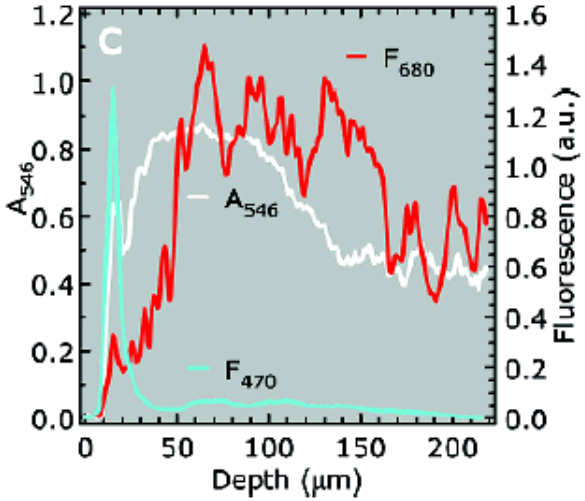



Anthocyanins

Chlorophyll

Skin (grape berry exocarp):
Single layer of clear epidermal cells
Six hypodermal layers with anthocyanins



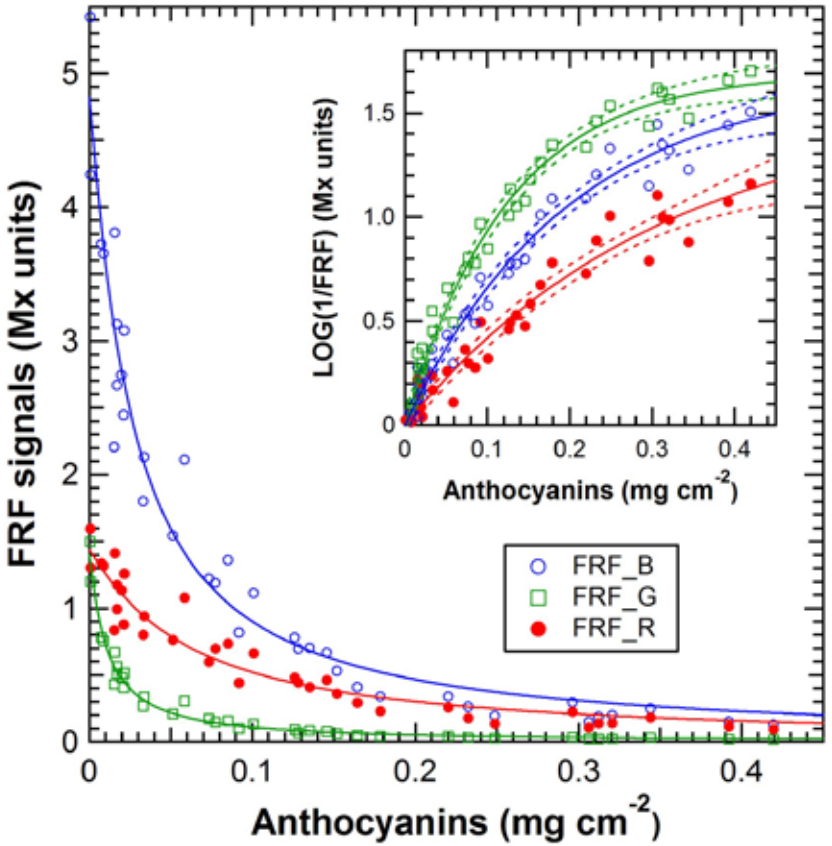

Multiplex sensor

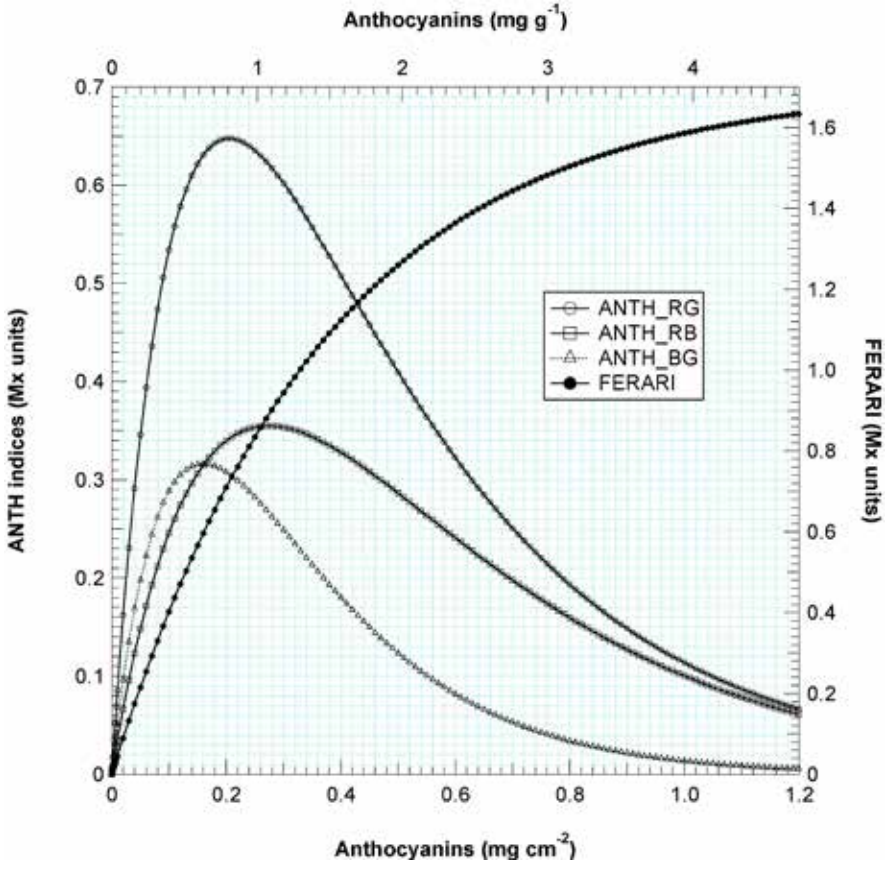
# Multiplex berry ANTH: a quantitative non-linear response

signals

*Ben Ghozlen et al. (2010) Sensors, **10**:10040*

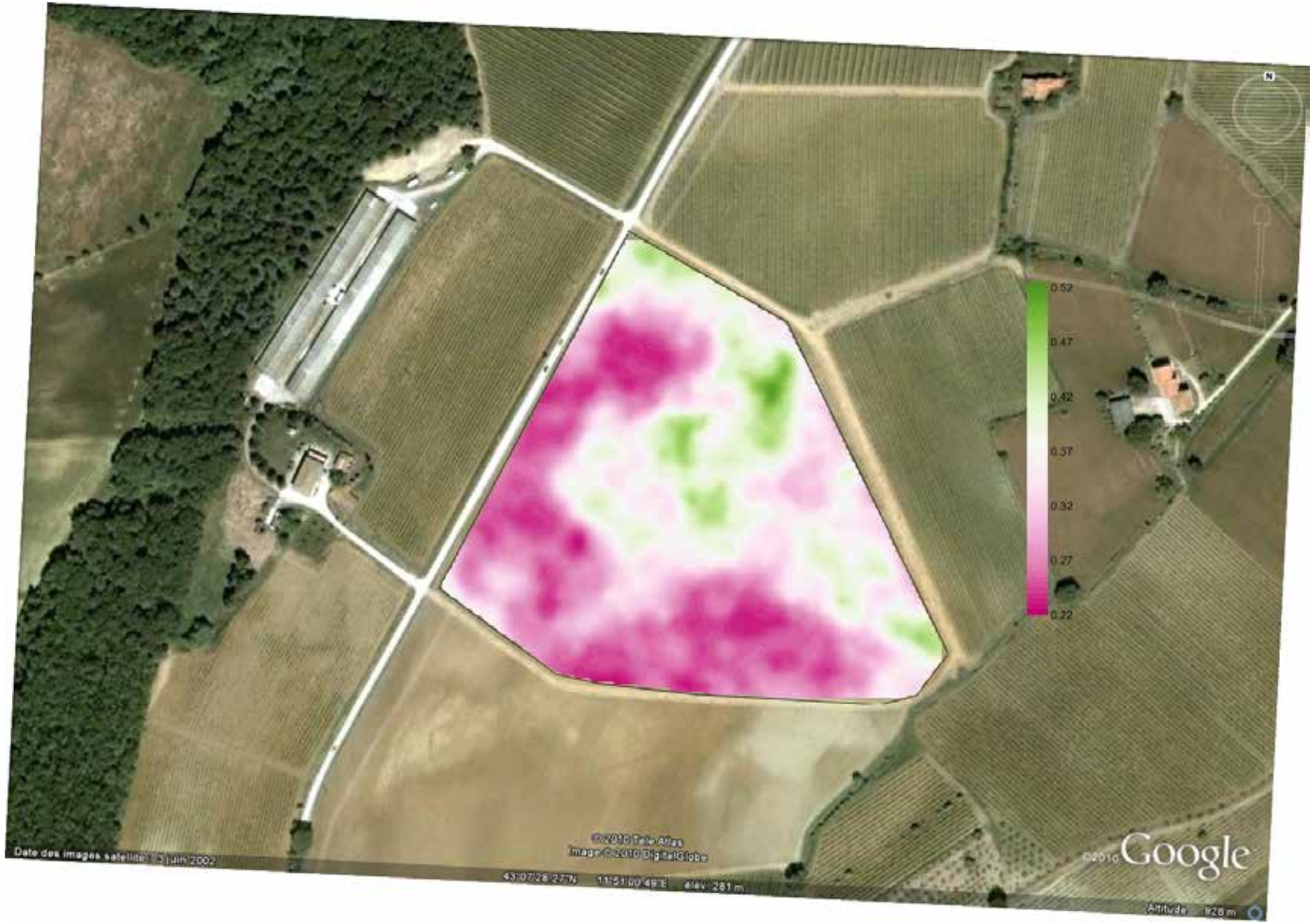
ratios




$$ANTH\_RG = A[\exp(-a_R\ Anth) - \exp(-a_G\ Anth)] + \log(\gamma_{RG})$$

# Grape-quality selective harvesting (Tuscany)



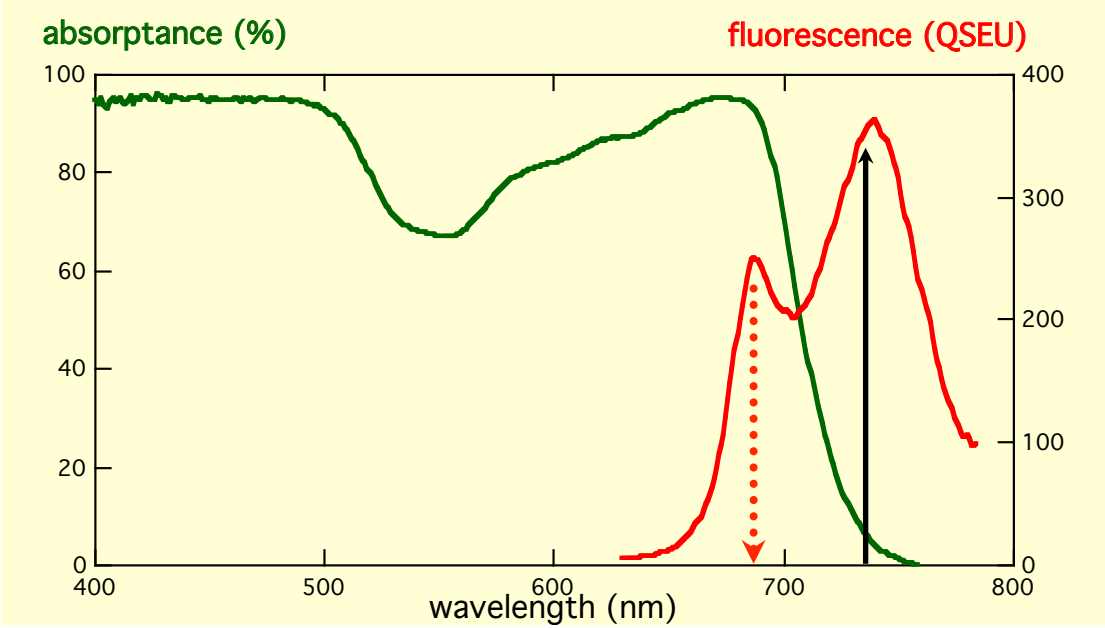
# The chlorophyll fluorescence emission ratio (SFR)

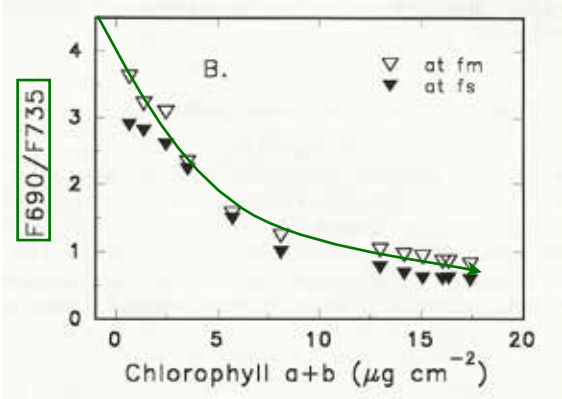
**The of use chlorophyll fluorescence to estimate the content of leaf chlorophyll content**

A method based on fluorescence reabsorption

Simple fluorescence emission ratio (SFR)

# Chlorophyll estimation from fluorescence reabsorption




*Babani et al. (1996) JPP, **148:** 471*

685 735

RF FRF

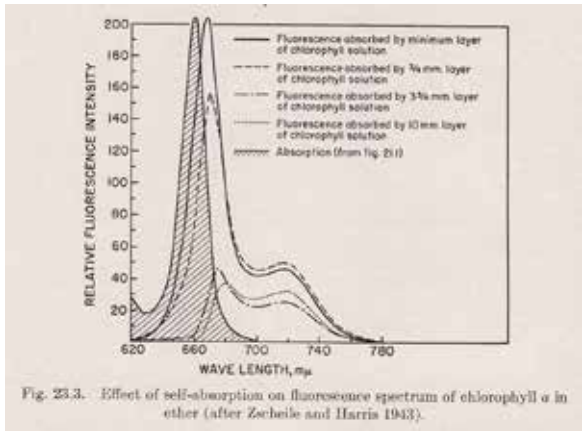
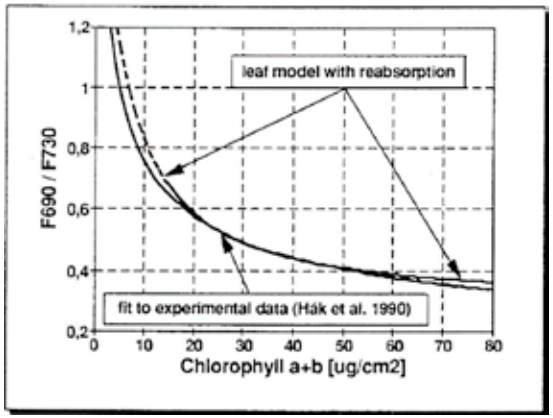
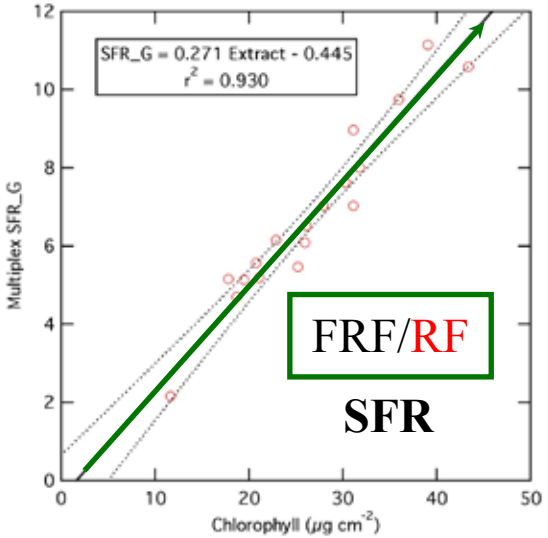



Fig. 23.3. Effect of self-absorption on fluorescence spectrum of chlorophyll a in ether (after Zscheile and Harris 1943).

*Rabinowitch (1951) Book*



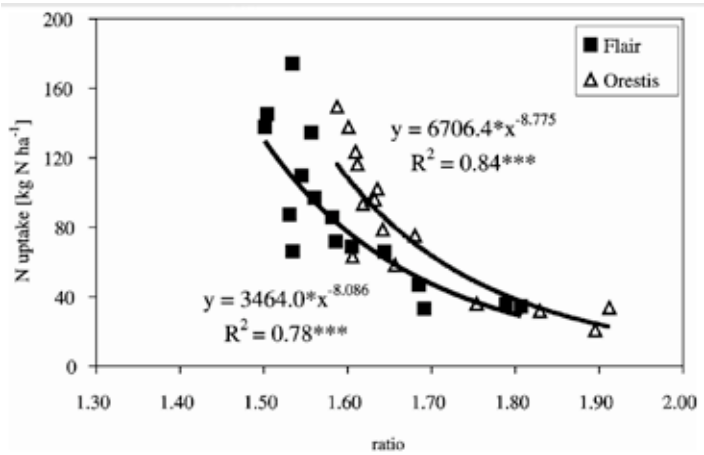

*Dahn et al. (1992) EARSeL Adv. Remote Sens., **1:** 12*




*Tremblay et al. (20012) Agron. Sust. Develop, **32**: 451*

# Tractor mounted sensors

maps ?




Laser-N-Detector (Planto)

*Schächtl et al. (2005) Prec. Ag.* ***6:*** *143*

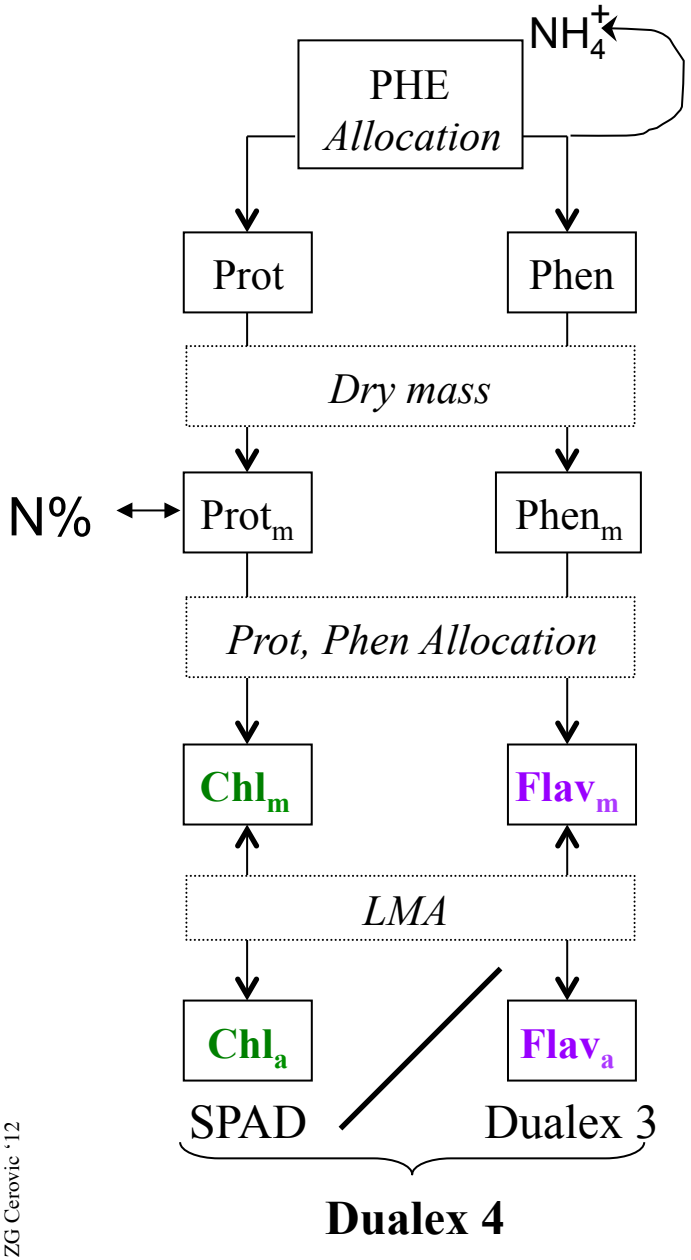

MiniVeg (Fritzmeier)

# The nitrogen balance index (NBI)

**Chlorophyll fluorescence linked to nitrogen nutrition**
Based on the **chlorophyll**/**flavonoid** ratio (NBI)
Dualex sensor

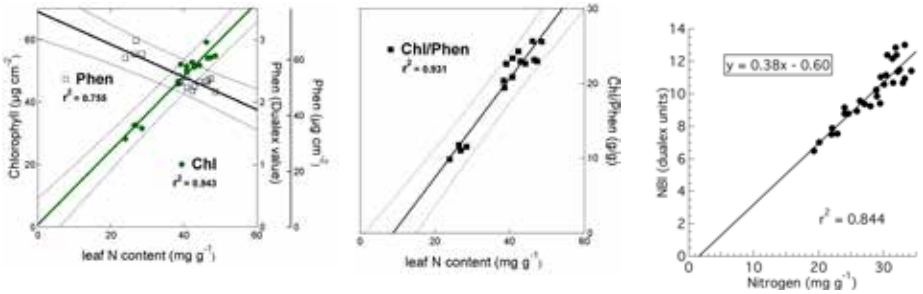
Based on a combined fluorescence spectral ratio (NBI)
Emission ratio (SFR)/Excitation ratio (FER)
FRF_UV/RF_G (NBI Patent)
Multiplex sensor

# The Chl/Flav ratio: Nitrogen Balance Index (NBI)

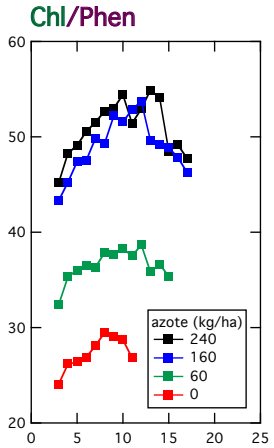



## Three Beneficial effects

**1.** The **opposite dependence** on nitrogen increases the dynamic range




**2.** The **parallel dependence** on leaf age decreases leaf position influence




**3.** The ratio of **two surface-based measurements** avoids the influence of LMA

# Optical decision support tools

*leaf-clip*

**Dualex**:

Chlorophylls

Flavonols

*proximal sensor : leaves and grapes*

**Multiplex**:

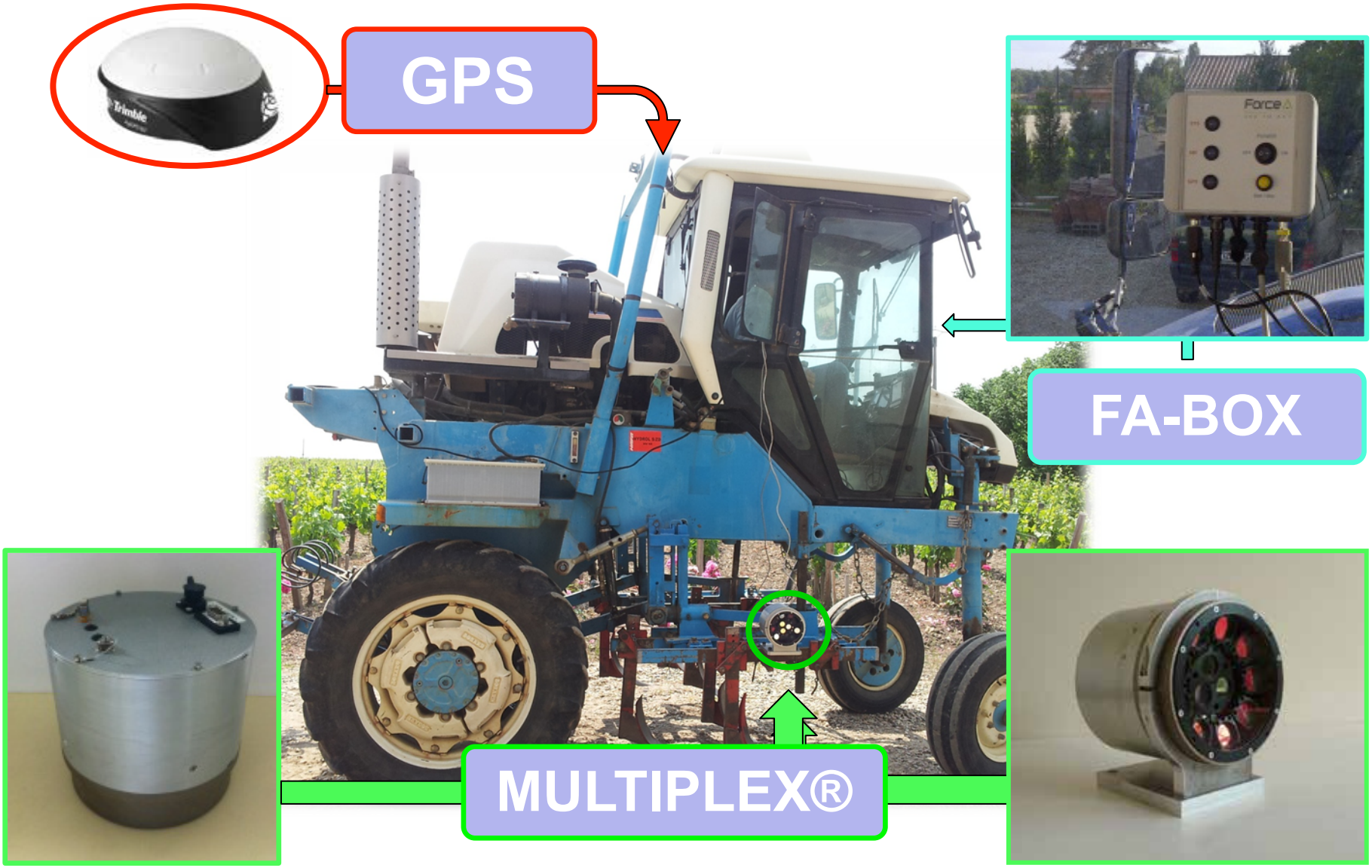
Chlorophylls

Flavonols

Anthocyanins

(Stilbenes)

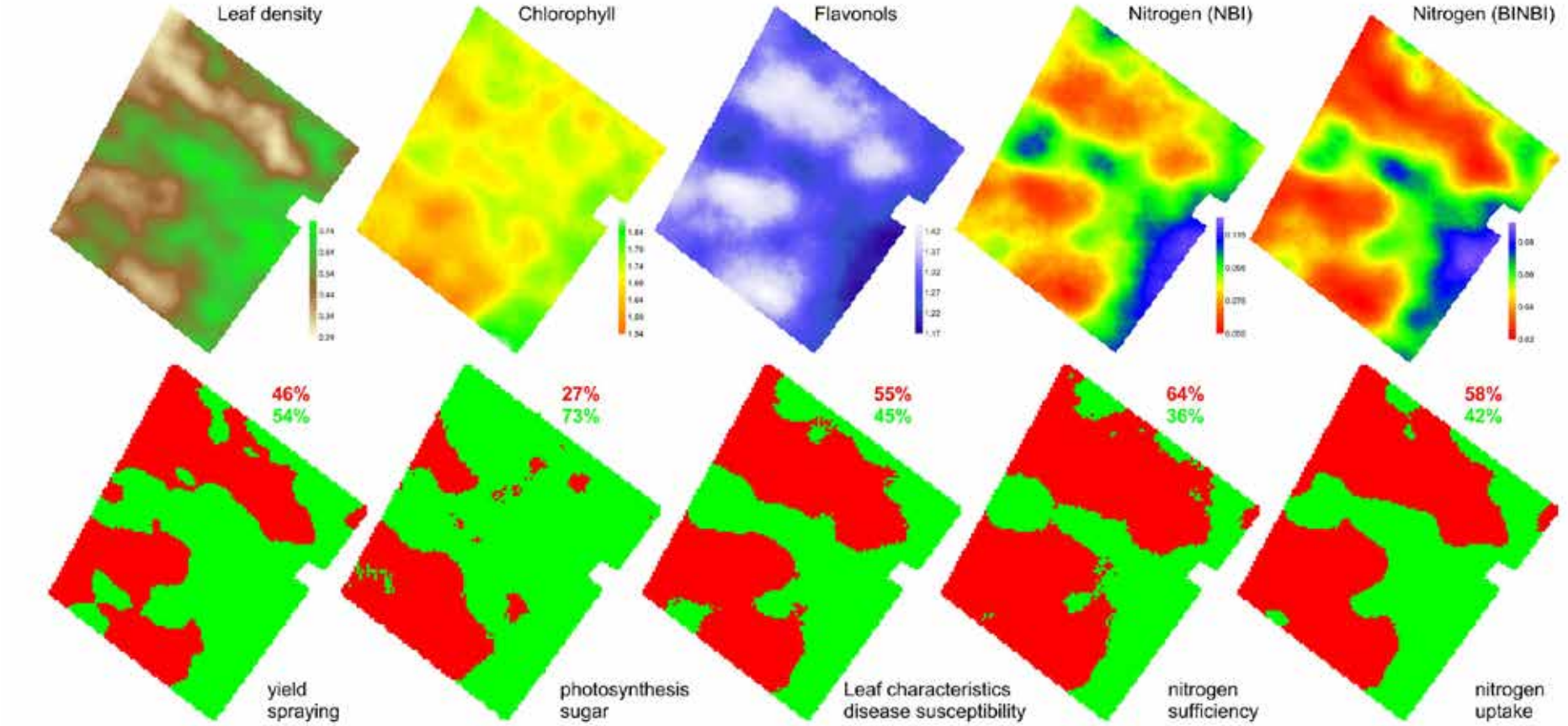
# Tractor mounted sensors for fluorescence-based vineyard mapping



# Spatial heterogeneity – Plot Zoning – Five-info maps

*Shoot number* *Shoot vigour* Vegetative expression

NFI ( Chl / Flav ) = NBI NFI*NBI




Gap fraction
LAD
LAIsv

Nitrogen
Surface-
based

Light
Exposure

Nitrogen
Mass-
based

Total
Nitrogen
per vine

# UV-induced blue fluorescence (BGF) (VBF)

**Autofluorescence of hydroxycinnamic acids**
Ferulic acid bound to the cell walls (veins)

**The blue-to-red emission ratio (BRR & BFRR)**

**UV-B-induced violet-blue fluorescence (VBF)**

# Autofluorescence of wheat leaves under UV-excitation

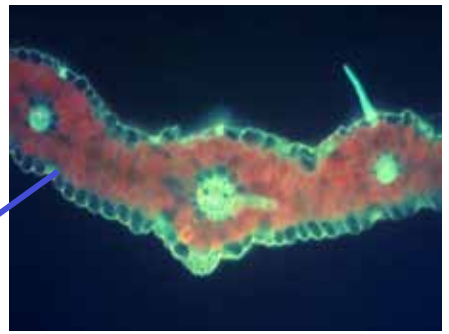
Variable ChlF
***Photosynthesis***

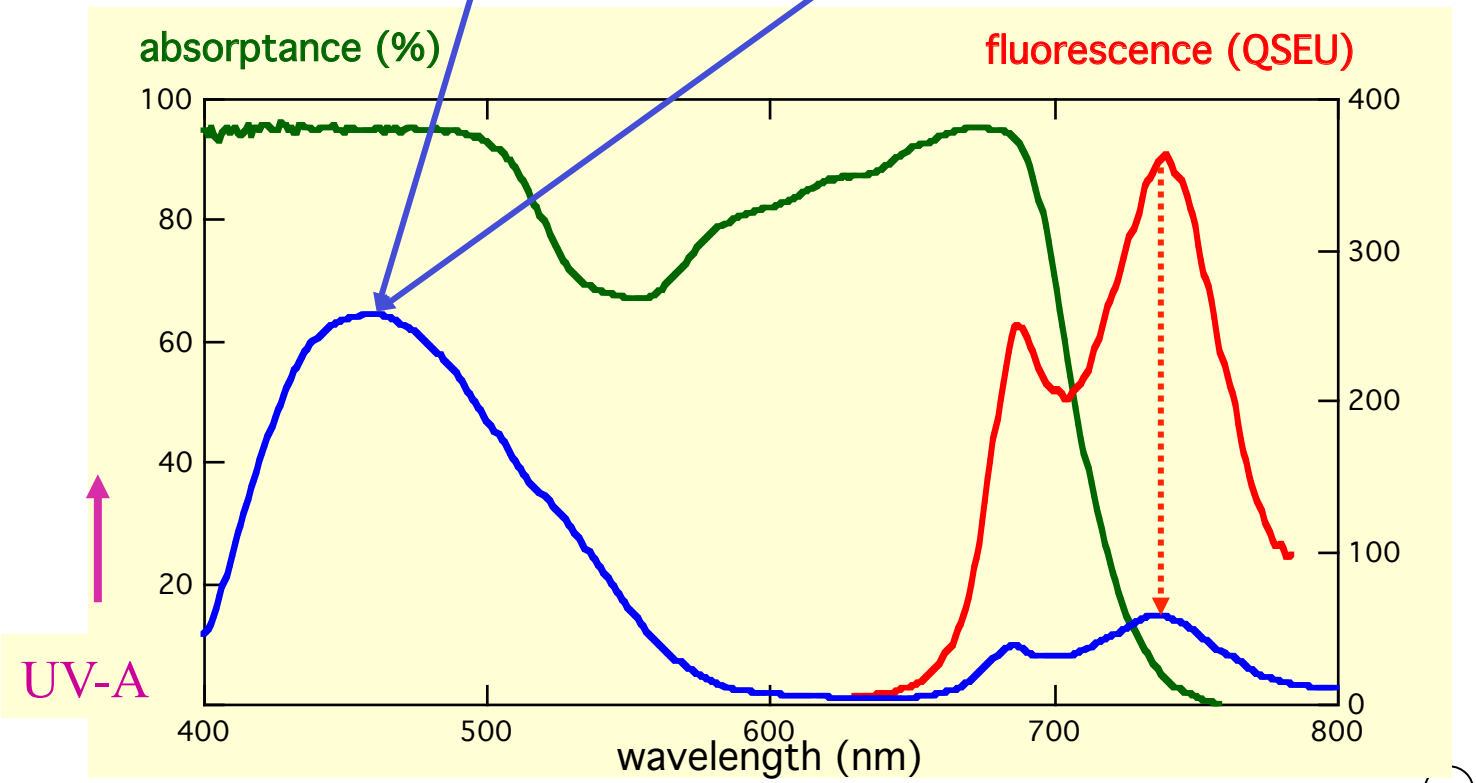
SFR
***Chl content***

FER
***Flavones***
***Flavonols***

NBI
***nitrogen***

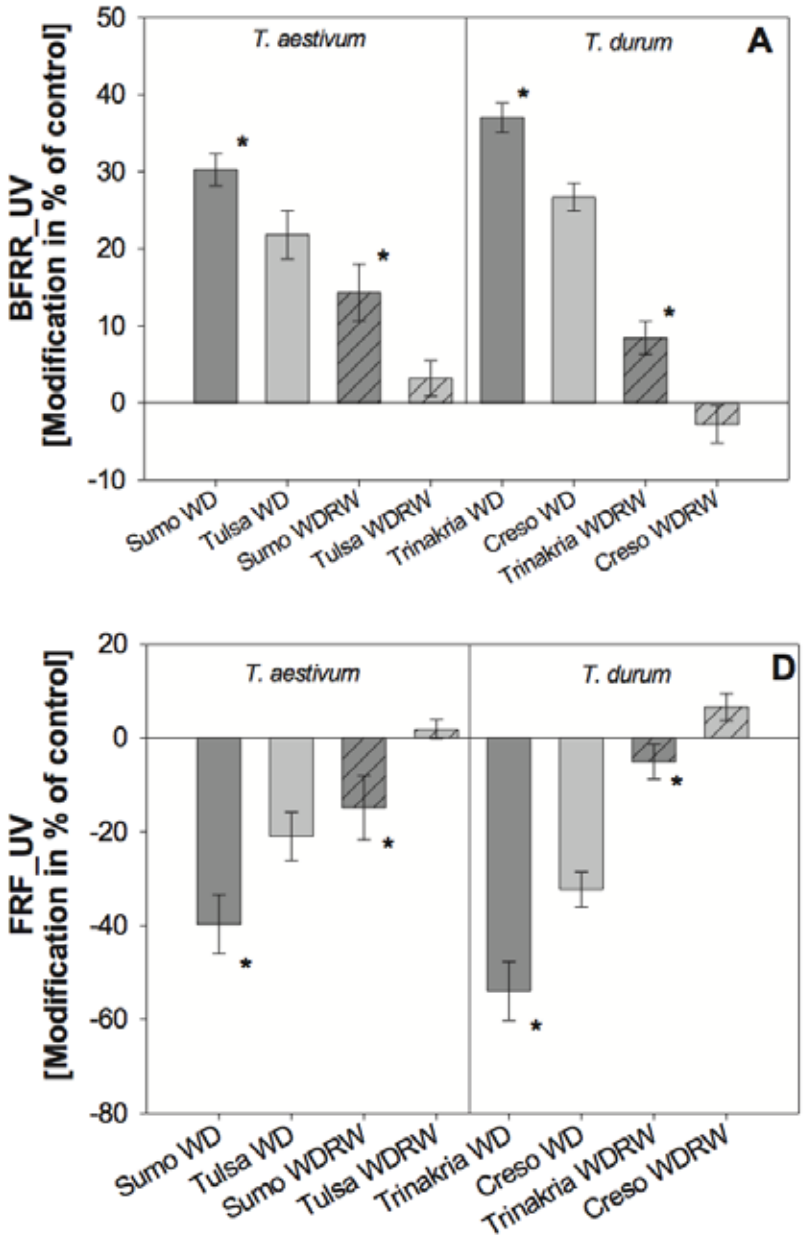
BGF (BFRR)
***Structure***
***Water stress***





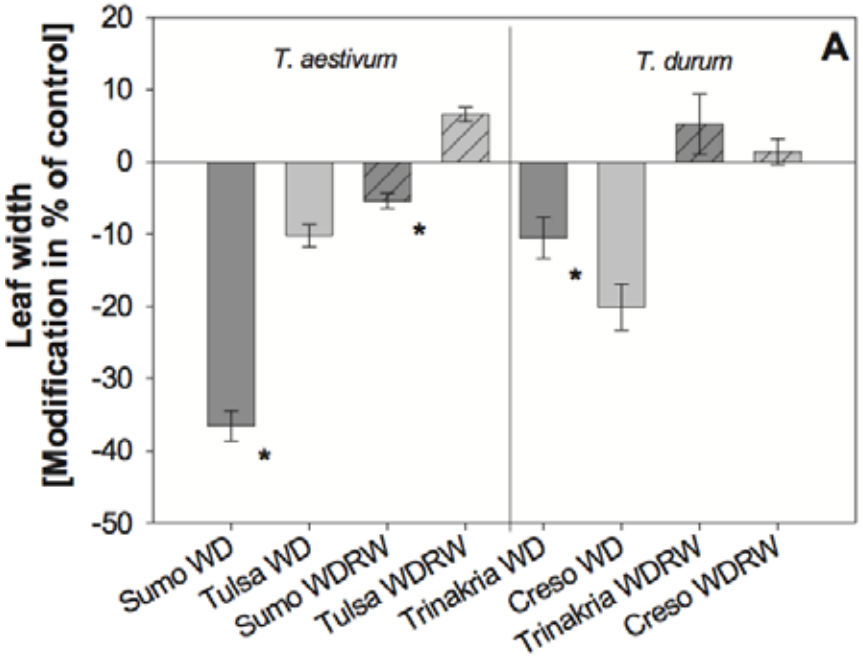
# Sensing of water stress by the blue-to-far-red emission fluorescence ratio

BFRR




WD: under water deficit

WDRW: water deficit then re-watered




*Bürling et al. (2013) Environ. Exp. Bot.* ***89****: 51*

# UV-induced plant blue-green fluorescence

- Temperature
- Redox state [NAD(P)H]
- Water stress
- Leaf development
- Nutrition

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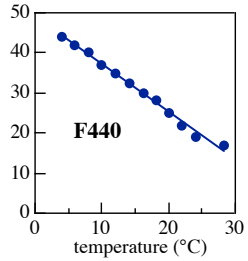
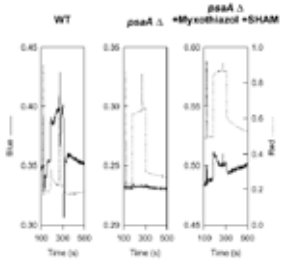
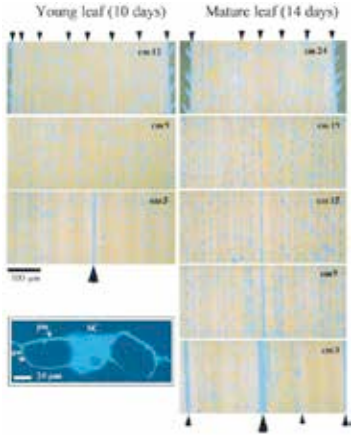
- Pathogen on leaf
- Leaf response
- Field infection




*I. Goulas (1992) thesis*



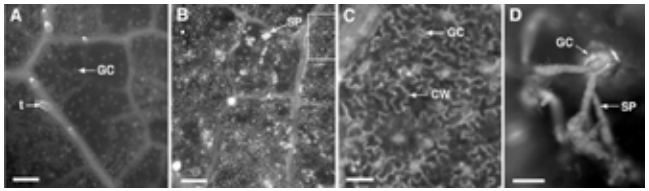
*Cournac et al. (2002)*
*Plant Physiol. **129**, 1921*




*Meyer et al. (2003)*
*J. Exp Bot **54**, 757*

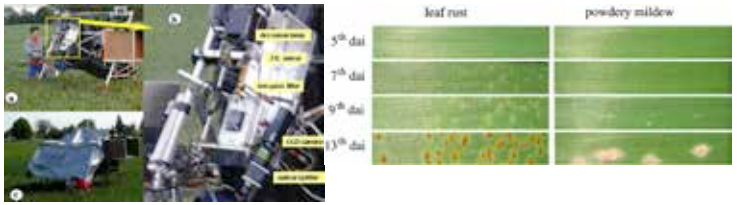
*cf. Chappelle et al. (1984)*
*Appl. Optics **23**, 134*


*Z.G. Cerovic (2005)*
*unpublished*




*Poutaraud et al. (2007)*
*J. Agric. Food Chem. **55**, 4917*


*G. Latouche (2010)*
*unpublished*




*Kuckenberg et al. (2009)*
*Prec. Ag. **10**, 34*

*Bravo et al. (2004)*
*Ag. Eng. Intl. FP 04008*

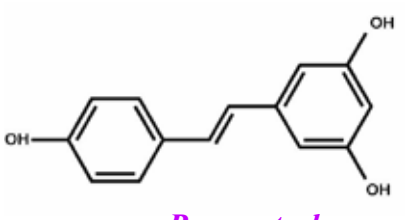
# Stilbenoid fluorescence

***UV-excited***
***blue-green***
***Fluorescence***
***BGF***

**HCA** **Fungi**

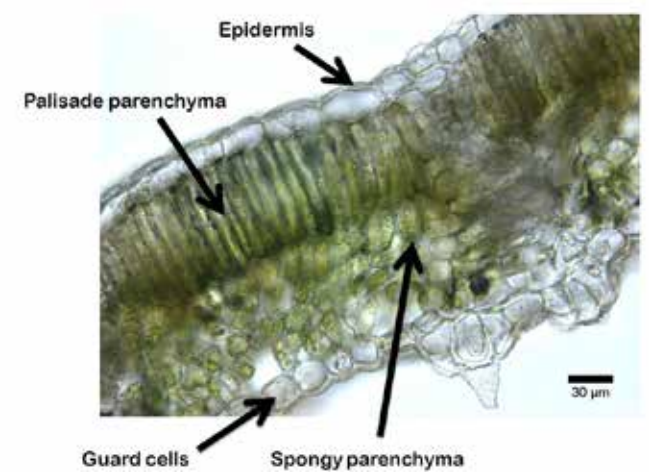
***UV-exited***
***violet-blue***
***Fluorescence***
***VBF***

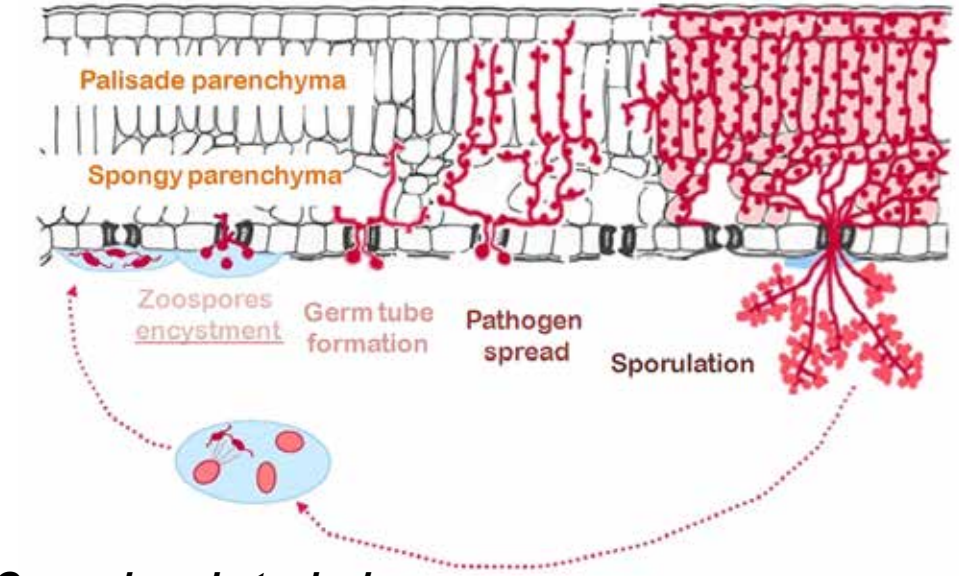
**stilbenes**

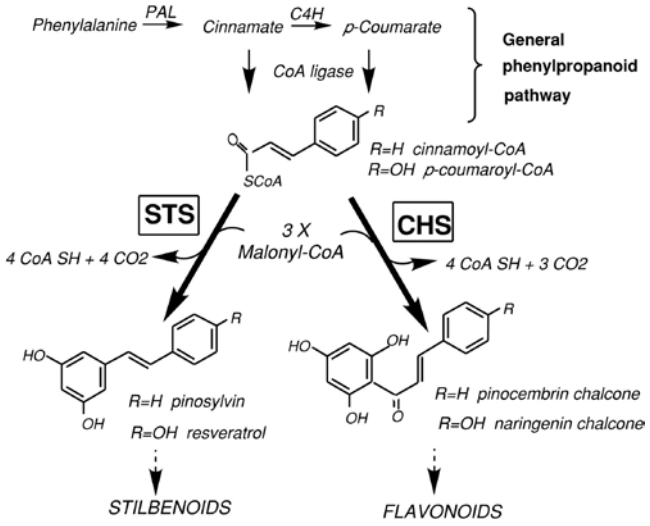



*Resveratrol*

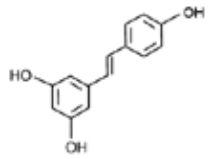
# *Plasmopara viticola* the infection agent of downy mildew

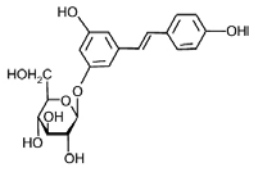


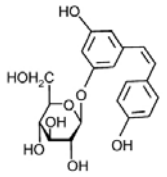




***Grapevine phytoalexins***
***Viniferins, stilbenoids, derivatives of resveratrol***


resveratrol


*trans*-piceid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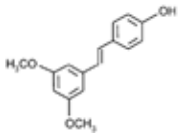
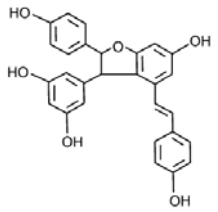
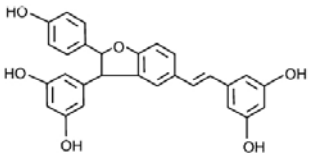

pterostilbene

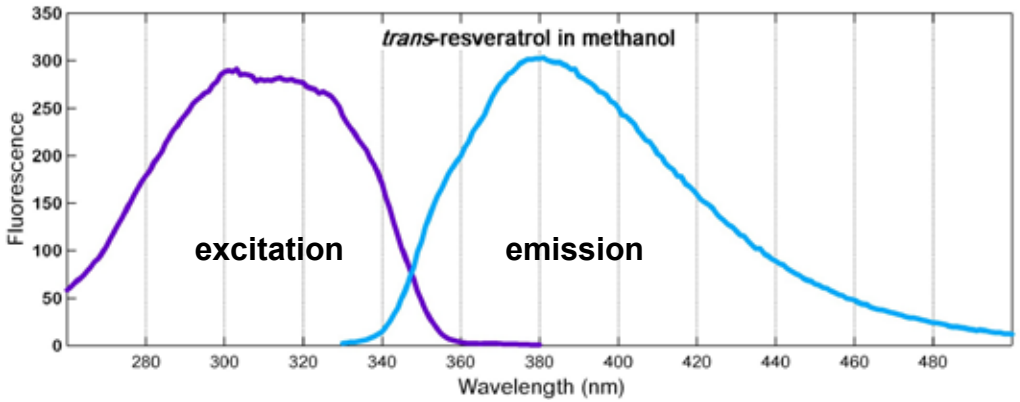

*trans*-ε-viniferin

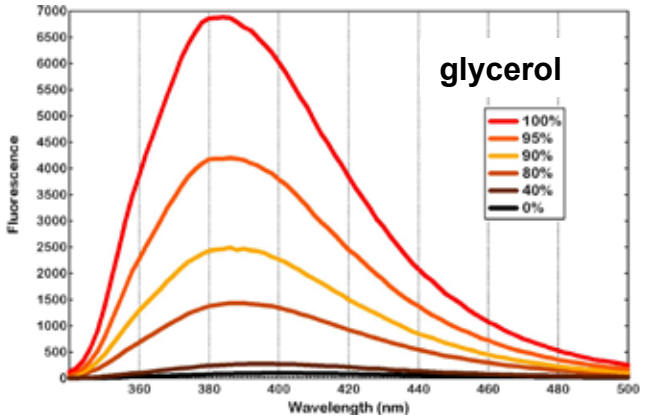

*trans*-δ-viniferin

# Fluorescence of stilbenoids

## Complete analysis of fluorescence: physico-chemical, microscopic et macroscopic

*Bellow et al. (2012) J. Exp. Bot.* ***63****: 3697*

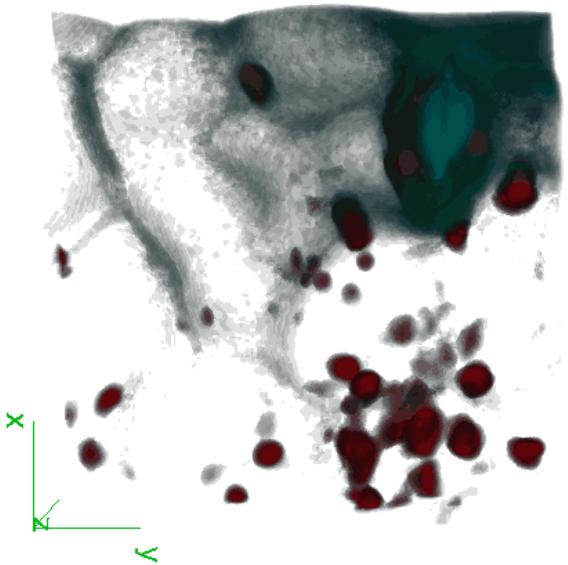


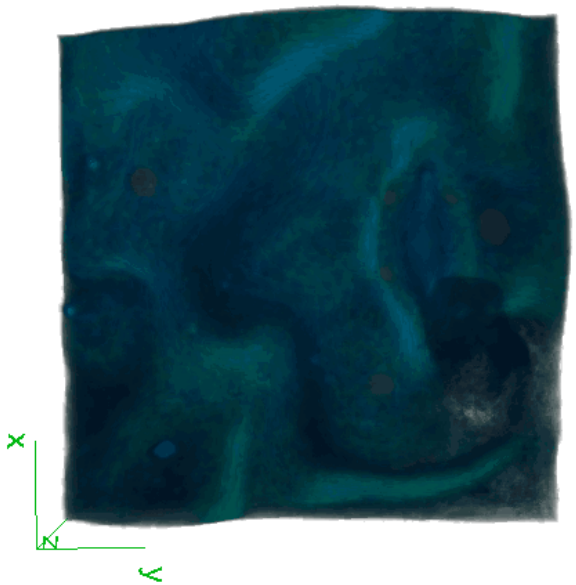




Control

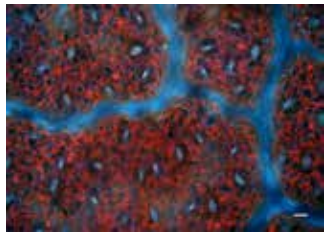
Cabernet Sauvignon

Inoculated

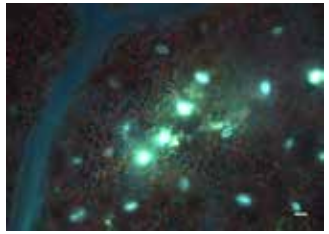




*Autofluorescence*



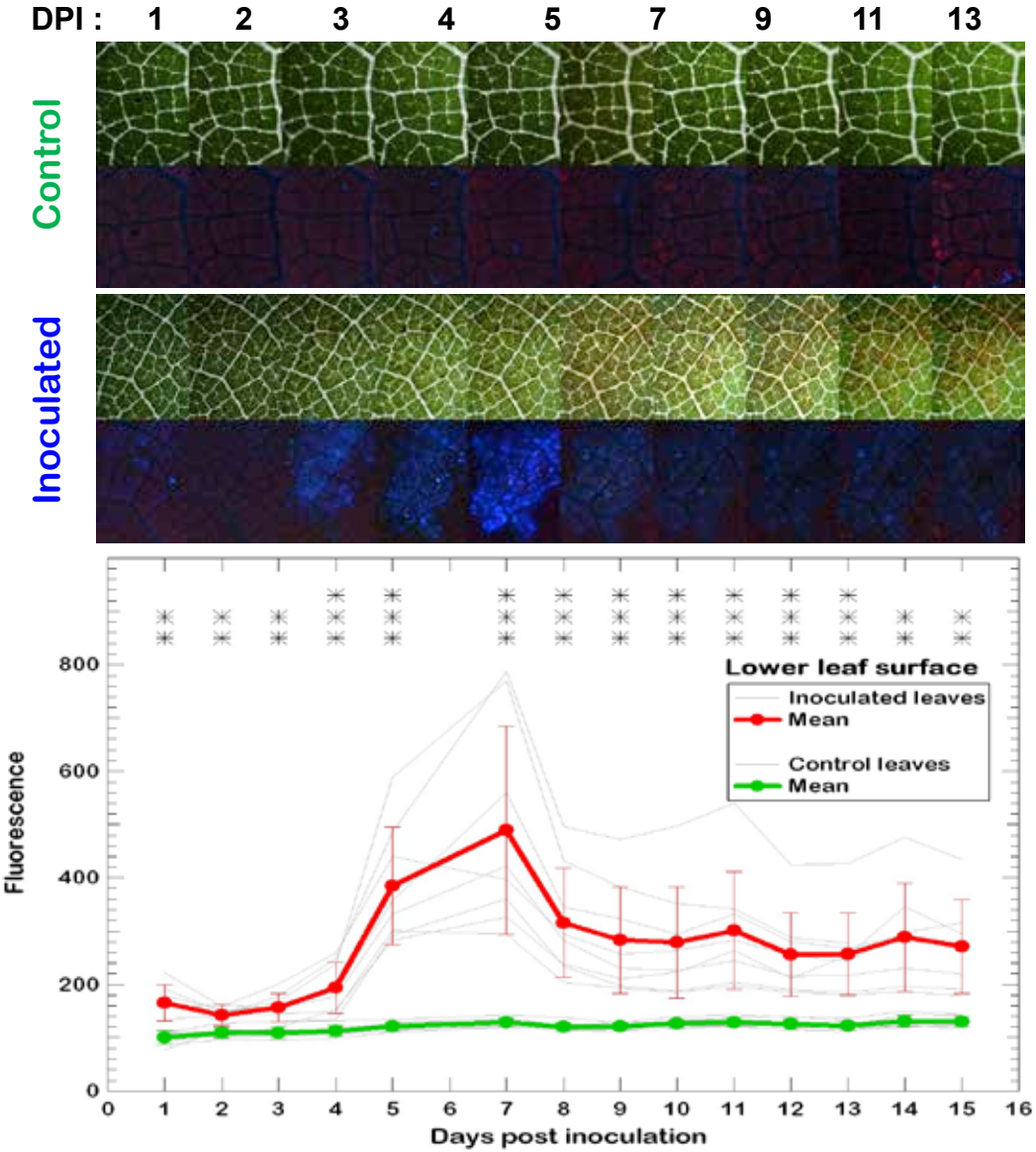
*control*



*infected*

Bellow *et al.* 2012

# Kinetics of the infection



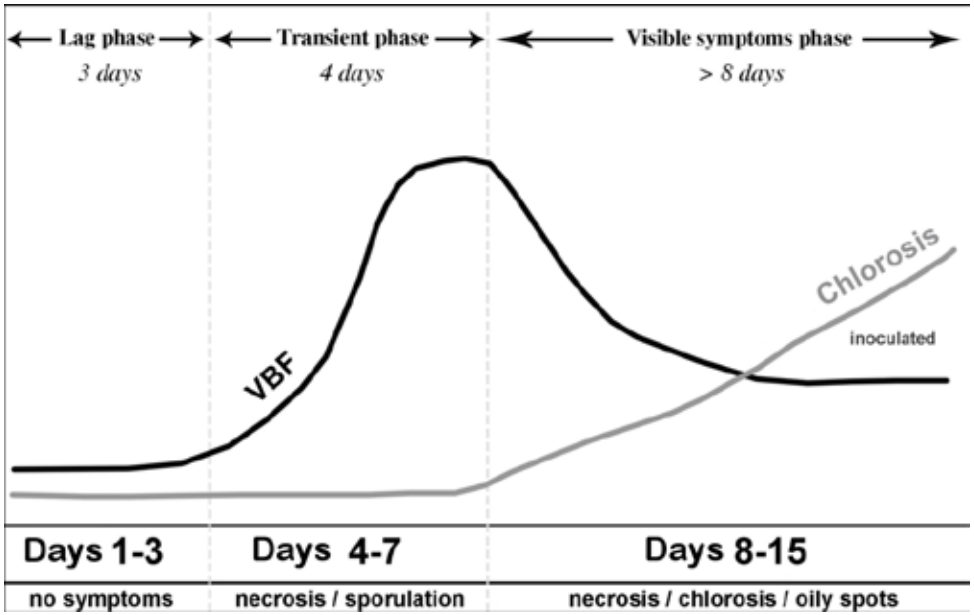

**Excitation filter :** 340/26
**Emission filter :** 371 nm long pass

**Macroscope Nikon AZ100**


**Proximal detection Mx330**

# Phytoalexins as disease markers

Mounted Multiplex for hot-spot detection




Grapevine
*stilbenoids*

Peanut
*stilbenoids*

Sunflower
*coumarins*

**Other crops Phytoalexins**

# Plant Biophotonics team

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Fermin Morales (Zaragosa)
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